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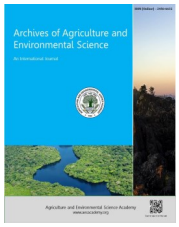

ORIGINAL RESEARCH ARTICLE

Taxonomic significance of stem and petiole anatomy of three white varieties of *Vigna unguiculata* (L.) Walp.

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ABSTRACT

Anatomical studies were carried out on the stem and petiole of three white varieties of *Vigna unguiculata* L. (Walp.) belonging to the family Fabaceae consumed in Awka Anambra State Nigeria. The structures of the stem and the petiole showed the basic structure of a dicotyledonous plant. The transverse section of the stem and petiole consists of epidermal and collenchyma layers, parenchyma cells and stele (vascular bundles, secretory cells and pith); however there were differences in shape and position of the vascular bundles. In the stem, this bundles are located on a continuous ring but in the petiole are cutting and divided into two large adaxial and three abaxial bundles forming main foliar trace, which above it lie laterally a pair of secondary bundles. The relationships found in this study provide insights to the phylogeny of the species and revealed the importance of combined data from different taxonomic evidences to have clearer information on varietal relationships to enhance the delimitation of *Vigna species.*

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INTRODUCTION

Vigna unguiculata (L.) Walp belongs to the family Fabaceae. It is one of the most ancient human food sourc-es and has probably been used as a crop plant since Neolithic times. An annual dicotyledonous legume and a warm weather crop, well adapted to drier regions of the tropics like Nigeria, where other food legumes do not thrive well (Sankie *et al*., 2012). Nigeria is the largest producer and consumer of *V. unguiculata*, accounting for about 45 percent of its world's production (Lowenberg-Deboer and Ibro, 2008; Ndong *et al.,* 2012) while the whole of Africa accounts for about 75% (Brissibe *et al*., 2011). Four subspecies are recognized; of which three are cultivated (more exist, including *textilis*, *pubescens*, and *sinensis*). Anatomical characters are very useful in the determination of relationship in orders and genera and their features have played an increasingly important role in phylogenetic relationships. Stace (1984) discovered that trichome anatomy is of immerse significance in classification at levels from the circumscription of the family down to the separation of species and even varieties. In particular it has led to an improved tribal classification within the largest genus Combretum. Edeoga and Okoli (1997) carried out a research on Costaceae family observed differences on the features of vegetative anatomy and suggested a separate specific status for *Costus afer* and *Costus lucanusianus* which opposed the conspecific treatment given to them by previous researchers. In leguminoceae-Ceasalpinoideae, the unicellular and multicellular trichomes described in certain species of *Senna* tournex Mill and *Senna hirsuta* was diagnostic in the acquisition of these two types of trichomes (Edooga and Osakwe, 1996). In anatomical study of the transverse section of the root of *Capsicum* species, homodorminant anatomical features was reported which strengthens the affinity relationship in the genus *Capsicum* (Aziagba *et al.,* 2014). Ezeabara *et al*. (2013) also recorded affinity relationship in the study of stem anatomy of *Citrus* species. Type, size, shape, stella patterns, vascular bundles, rays, parenchyma, epidermal and phloem cells are some of the basic anatomical characters of well established taxonomic value (Sharma, 1993; Pandey, 2007). In view of this, anatomy has been a critical tool to Taxonomist in the classification and separation of taxa (Illor *et al.,* 2011). Therefore, this research was to investigate the transverse section of the stem and petiole of three white varieties of *V. unguiculata* (*Var.* Potiskum, Sokoto guzo and Iron beans) popularly called cowpea to ascertain the use of stem

and petiole micro morphology in the identification and classification of *Vigna species* which will be useful in understanding the affinity relationship that exist among the genus *Vigna*.

MATERIALS AND METHODS

Procurement of materials: The seeds of *V. unguiculata* for this study were sourced from various markets in Anambra state where there were major distributors from the North. (Eke Awka, Aforigwe, Nkpor, Ose okwodu) Tags bearing numbers and alphabets were used for the identification of the places and varieties collected. This was authenticated by a Taxonomist Prof C.U. Okeke and Aziagba, B.O. of the Department of Botany, Nnamdi Azikiwe University Awka. Three white varieties; Potiskum, Sokoto guzo and Iron beans were then selected for the experiment. The voucher specimen was deposited at the Herbarium in the Department of Botany Nnamdi Azikiwe University Awka Anambra State Nigeria. Planting of the seed was between the month of August and September. Planting was done in rows and seeds spaced at 2 to 8cm in the row at the dept of 1 to 6cm deep ensuring good seed and soil contact. This was on complete randomized design (CRD). The stems and petioles for the study were collected thereafter at the flowering stage of the plant.

Anatomical studies: The stems and petioles of the three white varieties of *Vigna unguiculata* under study were collected from the experimental site and preserved in vials containing formaldehyde, glacial acetic acid and alcohol in the ratio of 1:1:8 respectively. The specimens were dehydrated in ethanol series (30% 50% 70% and 95%) each for 2 hours. The specimens were stored in absolute ethanol (99.6%) overnight to ensure complete dehydration of the stem and petiole specimens of the tree white varieties of *V. unguiculata.* The specimens were then cleared in 3:1, 1:1 and 1:3 ethanol/chloroform each for 3 hours. Anatomical Wax was melted at 70°C in an oven. The cleared specimens were then put in molten anatomical wax and alcohol and allowed to stay at 70°C for 24 hours for effective infiltration of wax into the specimen to replace the chloroform which was gradually lost by evaporation. Embedding was carried out after infiltration. Embedding of the stems and petioles of the varieties for this research was performed by smearing glycerin inside the clean molds and the molten wax poured into the molds. This was done carefully to ensure that the molten wax suits the type of sections cut. Cooling of the wax in the mould was to allow it to block. These wax blocks were brought out from the mould stuck on wooden holder and labelling of the specimens were done prior to the time for sectioning.

Wax blocks which was freed from the holder was trimmed and affixed on the sledge microtome. Sectioning was performed at 15-20 microns thickness for the entire specimen. The thin sections were fixed on clean slides already smeared with a thin film of egg albumen. The sectioned stems and petioles were stretched by passing them over hot plate. This also made them to become attached to the slides. Slides bearing sections of the stems and petioles were arranged in a slide rack and placed in an oven at 70°C to melt off wax from the sections. This lasted for 12 hours.

For staining, the sections of the stems and the petioles were dehydrated by passing slides across xylene and xylene/absolute ethanol series (3:1, 1:1 and 1:3 v/v), absolute ethanol, 95, 70,50 and 30% ethanol. The slides bearing the stems and petioles of three varieties were briefly immersed in water, then stained with 0.1% alcian blue and counter stained with 1% safranin. Stained slides were rinsed briefly in tap water dehydrated through the ethanol series and cleared across the xylene /absolute ethanol series.

Mounting of the slides bearing the stems and petioles of the three white varieties was carried out by placing one drop of Canada balsam on a clean slide and carefully covering the sections with the cover slip in a way that the Canada balsam will spread and covered the specimen sections overlaid by the cover slip. They were studied and photomicrographs taken with DSWC 230 Sony Camera. This method was the method of AOAC (1984).

RESULTS AND DISCUSSION

Results of the anatomy of the stems and petioles of the three white varieties Potiskum, Sokoto guzo and Iron beans *V. unguiculata* are presented in Plates 1-6.

The transverse section of the stem of the three varieties appeared in ribbed form. The epidermal layers showed single layer of epidermis covered with rectangular cells and a thick cuticle. There was presence of ring pores which are round and appeared in multiple radial indicating growths. Apotrachial parenchyma in var. potiskum was banded and paratrachial were also banded, possessing oval cells and thin walls inside the cortex, this was also observed in var. iron beans and sokoto guzo. Collenchyma was present near the epidermis. There was presence of xylem and phloem. Xylem is composed of protoxylem toward the plant center and metaxylem at the apical side of plant. The secretory cells were located very close to the phloem. These bundles are relatively of different sizes and numbers. The vascular bundles were more in number in Potiskum and Iron bean. There was also presence of large pith in the stem center which consists of polygonal parenchymatous cells which tend to decrease in size towards the periphery. Intercellular spaces were small and visible in the three white varieties and vessels of different sizes (Plates 1-3). The slight variations and similarities in the number of vascular bundles, ring pores, parenchyma cells and collenchyma cells in the stem showed intra and interspecific relationship among the varieties. Ajuru and okoli (2013) noted variation in the layers of collenchymas in Mormodica species as a distinguishing character. In the petiole, the upper and lower epidermis of the three varieties is covered with thick cuticle. These layers are single-layered with rectangular cells .The stomata observed are paracytic as in Fabaceae family which consist of guard cells typical of kidney shape. These stomata occur on both the lower and upper epidermis but most frequently on the lower surface. The mid rib is well developed. The xylem and phloem seen were collateral on the three varieties. Xylem is towards the upper side while phloem is on the

lower side. These variations are diagnostic and could be used to differentiate the varieties. Among these characters the parenchymatous layers and number of vascular bundles which are more in number in Potiskum and Sokoto guzo and of various sizes at the apical portion of the petiole was found to be constracting. Metcalfe and Chalk (1950) and Abbas *et al.* (2006) have noted that the number of petiolar bundles is a reliable delimiting character among dicotyledonous plants. The character has been used in the taxonomy of many plant families. Norani *et al.* (2016) revealed the use of petiole bundle as a taxonomic tool in the identification of some species of the tribe Dipterocarpeae. Devades and Brck (1972 and Ataslar (2004) also reported that vascular bundles form a continuous ring, this is in agreement with Ataslar (2004) that vascular bundles are also seen in many points of the adaxial and abaxial surfaces of the leaf. Shaheen (2006) reported variations in the number of secondary vascular bundles in the petioles of some mimosoid species, and thus used as distinguishing characters among the taxa. The presence of trichomes varies among the varieties (Plate 3-6). These differences suggested moderate affinity in the white varieties. The variations in the position of the rays and pore sizes and numbers strengthen the reliability of anatomical characters in systematic botany as stated by Ayensu (1970). Scientific importance and implications of anatomical features in different groups of plants has been reported in many literatures. They include Dioscoreaceae, where certain anatomical features were used in the characterization of *Dioscorea alata* (L.) and *Dioscorea smilacifolia* (L.) (Edeoga, 2002). Edeoga and Okoli (1997) conducted their research on Costaceae family observed differences in the features of vegetative anatomy and suggested a separate specific status for Costus afer and Costus lucanusianus which opposed the conspecific treatment given to them by previous researchers. In Leguminosae-Caesalpinoideae, the nature of unicellular and multicellular trichomes described in certain species of Senna Tourn ex Mill and Senna hirsuta was reported to be diagonostic in acquisition of these two types of trichomes (Edeoga and Osawe, 1996). The cambium initials were observed in the primary vascular bundle between the xylem and phloem at the basal part of the stem. These tissues are seen in many fabaceae families. There are many unicellular trichomes on the petiole transverse section. The morphology and density of leaf trichomes vary considerably among plant species this was reported by Edeoga and Osakwe (1996) and they also stated that it may also vary among populations and within individual plants. Thus, the structure of the trichomes can range from unicellular to multicellular and it can be straight, spiral, hooked branched or unbranched. (Edoga and Osakwe, 1996). The presence of collenchyma cells in layers and position are of taxonomic importance (Shaheen, 2006, 2007). In general, the anatomical structures of the stem and petiole of the three white varieties were very important and could be used in the diagonostic key of the taxa at all taxonomic levels. The variations seen in some of the anatomical structures can be attributed to environmental conditions or gene breakdown observed in some angiosperms while the similarities were more accurate showing the three varieties are phylogenetically related. Although no character is absolutely immutable, some are fixed than others and it is on those that are less plastic that the systematic anatomists rely because they are not really affected by environmental conditions (Guerra and Nogueira, 1990). Therefore comparative plant petiole and stem anatomy have been observed to be reliable in plant taxonomy and systematic of many angiosperm species.

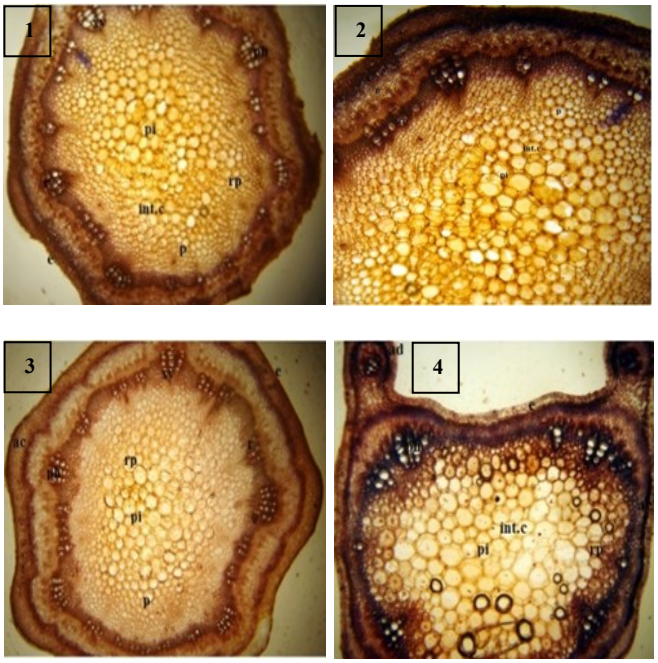

Plate 1-3. *Transverse section of stem of V. unguigulata (Var. Potiskum, Sokoto guzo and Iron beans × 40), respectively.*

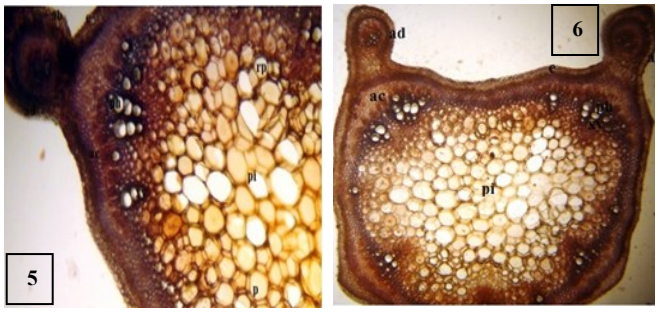

Plate 4-6. *Transverse section of Petiole of V. unguigulata (Var. Potiskum, Sokoto guzo and Iron beans × 40), respectively.*

Conclusions

The present study concluded that there is need for additional ways to identify morphological similarity between varieties of V. unguiculata (L.) and stem and petiole anatomy is one of them because it revealed the intra and interspecific relationship in and between the three white varieties of V. unguiculata. A dichotomous key to aid the identification of Vigna varieties is provided.

A dichotomous key for the identification of white varieties of V. unguiculata

1 Stem of white varieties	2
1 Petiole of white varieties	3
2 Shape of ring pores round	3
2 Shape of ring pores scattered	Sokoto guzo
3 Apotrachial parenchyma Terminal	Sokoto guzo
3 Apotrachial parenchyma banded	2
4 Collenchyma layers single	5
4 Collenchyma layers 1-2	Iron beans
5 Intercellular spaces normal	7
5 Intercellular spaces narrow and aggregate	Sokoto guzo
6 Number of vascular bundles stem 22-28	
6 Number of vascular bundles petiole	10-14

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